

Supplementary Information for

Optimizing Tenogenic Differentiation of Equine Adipose-Derived Mesenchymal Stem Cells (eq-ASC) Using TGFB3 Along with BMP Antagonists

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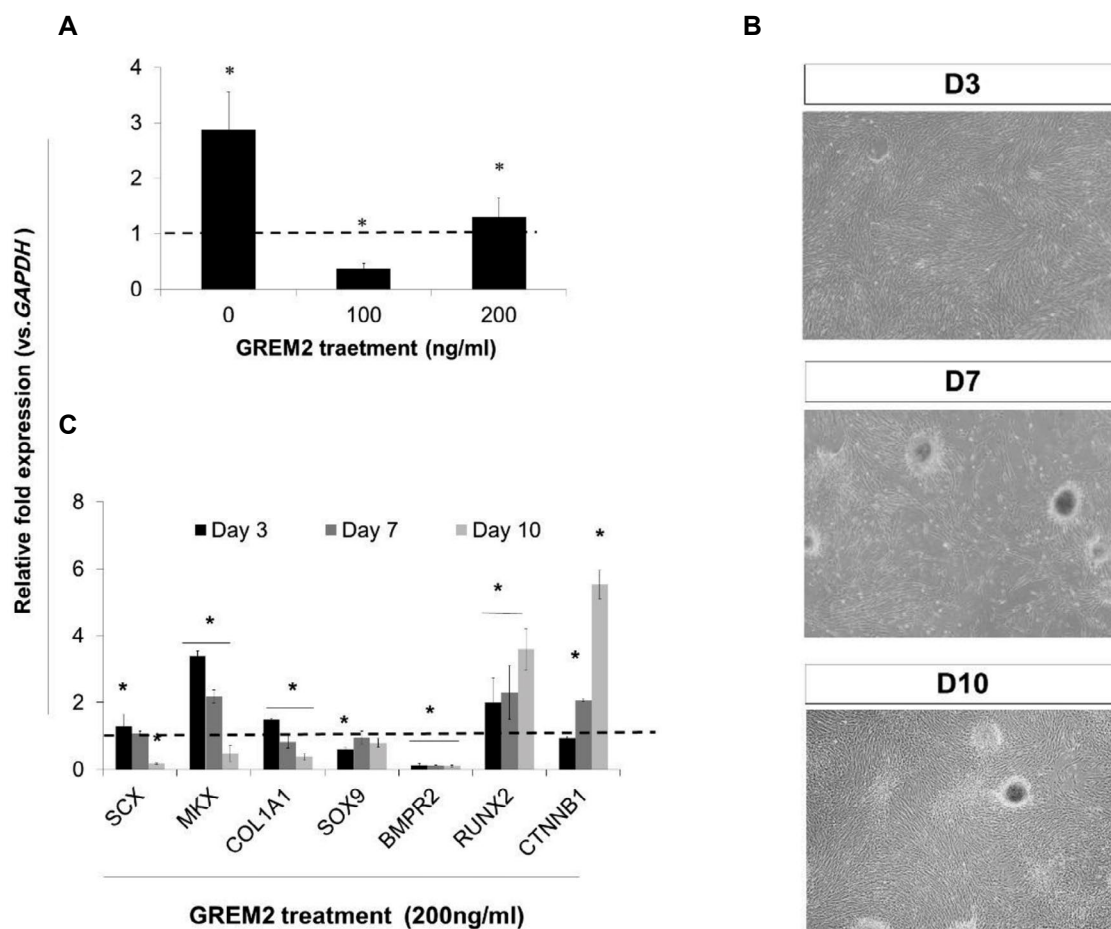


Fig.S1: Concentration-dependent effect of GREM2 on tenogenic differentiation. **A.** eq-ASCs treated with 100 and 200 ng/ml GREM2 showed a little increase in expression of SCX in response to 200 ng/ml at day 3 while decreased compared to control group (1.3 vs. 2.8). **B.** Morphological changes and qRT-PCR analysis for tenogenic-related genes (*SCX*, *MKX* and *COL1A1*), and **C.** Osteogenic-related genes (*CTNNB*, *RUNX2*, *BMPR2*) of eq-ASCs in response to 200 ng/ml GREM2 on three-time points are shown (day 3, 7 and 10) (scale bar: 200 μ m). Data were normalized to *GAPDH* and presented as mean \pm SD. *; Present significant changes vs. day 0 untreated cells (dashed line), $P < 0.05$ and QRT-PCR; Real-time polymerase chain reaction.

Table S1: Culture medium composition for induction of tenogenesis in eq-ASCs

Medium	Description
Ctrl	Basic medium: DMEM-high glucose, 10% FBS, 1% P/S
T	Basic medium+TGF- β 3 (2.5 ng/ml)
T/G	Basic medium+TGF- β 3 (2.5 ng/ml)+GREM2 (200 ng/ml)
T/G/S	Basic medium+TGF- β 3 (2.5 ng/ml)+GREM2 (200 ng/ml)+SOST (1250 ng/ml)

Ctrl; Control, T; Treatment with TGF- β 3 (2.5 ng/ml) for one day, T/G; Pretreatment with TGF- β 3 (2.5 ng/ml) followed by GREM2 (200 ng), and T/G/S; Pretreatment with TGF- β 3 (2.5 ng/ml) followed by GREM2 (200 ng/ml) and SOST (1250 ng/ml).

Table S2: Primer sequences which were used for real-time polymerase chain reaction

Gene	Accession Number	Primer sequence (5'-3')	Amplicon length (bp)
<i>GAPDH</i>	NM_001163856.1	F: GTGCTGAATATGTTGTGGAGT R: AGAAGGAGCAGAGATGATGAC	104
<i>SCX</i>	NM_001105150.1	F:GAACGCCCAGCCCAAACA R:CATCCGCCTCTAACTCCGAATC	103
<i>MKX</i>	XM_014737017.1	F:AATAATCCCGTTCACCATCCTG R:TTTGCCCTGTCTTTCCCATCAT	196
<i>COL1A1</i>	XM_023652710.1	F:CGGGTTTGGAGGAAAGTCAGG R:ACGAGGTAGTCTTTCAGCAAC	140
<i>TNMD</i>	NM_001081822.1	F:TCTTCACTTCCCTACCAACGA R:AATAACCTCTCTCATCCAGCA	179
<i>SOX9</i>	XM_014736619.1	F:ATTCCAAGACAGCAACATTCG R:ACACGGTTCTCCATCATCCT	157
<i>CTNIB1</i>	NM_001122762.1	F:ACTGTTCTTCGTGCTGGTGAC R:AGTGGGATGGTGGATGTAGGA	163
<i>BMPR2</i>	XM_014732300.1	F:GACTCTGCTACTCTAACATCC R:TCGTTCAAGGGTAATTAAAGC	158
<i>RUNX2</i>	XM_005603968.2	F:ACGCATTCTAACCTATGTCAG R:GGGTAAGACAGACTAAAGGAC	133